Effects of Creatine Precursors in Arthritis

Clinical and Metabolic Study of Glycocyamine and Betaine

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• Twenty-two patients with arthritis received 5 gm. of glycocyamine and 20.16 gm. of betaine by mouth daily, in hospital and after discharge, for periods ranging from 13 to 42 days. A control group of 22 patients, indistinguishable from the treated group, were maintained on the same regimen and given placebos resembling the agents under study.

The condition of each patient was evaluated clinically before, during and after treatment, and x-ray and metabolic studies were made. No significant improvement attributable to the use of the drugs was observed.

IN A PRELIMINARY report Borsook[2] indicated that certain precursors of creatine, when given by mouth to patients with rheumatoid and gouty arthritis, were clinically beneficial in a small series studied. In subsequent publications further studies on these creatine precursors, betaine and glycocyamine, were reported.[3, 4, 12, 22]

This report concerns observations on 44 arthritic patients in U. S. Navy Hospital at Oakland, fifteen of whom continued to receive therapy as out-patients after discharge from the hospital. Glycocyamine* and betaine* were administered to half of the patients; the others were controls who were given placebos in a "double blind" clinical study.

MATERIAL AND METHODS

The 44 patients observed were male naval personnel and veterans admitted for the treatment of arthritis. Age, duration of disease, classification of functional capacity and any concurrent disease are shown for each patient in Table 1.

The study was designed as a "double blind" experiment, using the techniques described by Greiner and co-workers,[14] with some modification necessary for a study of arthritis. The patients were placed in two groups, and as far as possible each patient in one group was matched by a patient in the other group with disease of the same clinical type and degree of severity. The 22 patients in the control group received an inert placebo. The 22 patients in the treated group received 5 gm. of glycocyamine and 20.16 gm. of betaine divided into two daily doses while they were studied in hospital; the same dosage divided into four daily doses was given to patients on whom study was continued after they left the hospital. The agents being tested were indistinguishable from the placebo, and were not identifiable to the patient, the physicians, or the ward attendants.

All patients admitted to the study were placed on a weighed diet containing 2,500 calories with 90 gm. of protein, and including a calculated daily intake of 5.0 gm. of sodium, 3.1 gm. of potassium, 1.0 gm. of calcium, and 1.6 gm. of phosphorus. Liberal amounts of vitamin C were present in the diet.

Moderate activity about the hospital was encouraged. In addition to the agent under study only analgesics were given and no additional therapy other than simple physiotherapy was used.

The investigation as originally designed was to include study and observation on each patient for a period of 48 days. A six-day preparatory period was allowed to stabilize the subjects under the conditions of the experiment. The treatment period with either agents or the placebo was originally intended to be 32 days, followed by ten days of continued observation; but in order to observe the possible effect of a longer treatment period, some changes in the original schedule were made. Furthermore, study had not been completed on a number of patients when the investigation was terminated because it became apparent that no significant results were being obtained even in those patients under treatment for the longest period of time.

As patients were admitted for study, they were collected into groups to facilitate the completion of the laboratory work. In the first group, 18 patients were treated for 42 days; in the second, twelve for 37 days; in the third, five for 31 days; in the fourth, seven for 23 days, and in the fifth, two for 13 days.

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TABLE 1.—*General Features of Arthritic Patients Included in the Study.*

Age	Duration of Disease Yrs.	Mo.	Type of Arthritis and Concurrent Disease	Functional Capacity*
20	....	9	Rheumatoid	II
34	5	....	Marie-Strumpell	I
52	30	....	Mixed—osteoarthritis, Marie-Strumpell	II
54	5	....	Traumatic	III
42	7	....	Osteoarthritis	I
28	2	....	Rheumatoid	II
22	1	5	Rheumatoid	I
56	4	....	Osteoarthritis	I
51	....	14	Gout	I
22	....	10	Rheumatoid	I
25	5	....	Rheumatoid	I
58	8	....	Gout	II
59	20	....	Mixed—osteoarthritis, rheumatoid	IV
48	7	....	Rheumatoid	IV
23	....	14	Rheumatoid	II
57	7	....	Rheumatoid; also arteriosclerotic heart disease with mild congestive failure	III
55	24	....	Mixed—osteoarthritis, rheumatoid	II
51	16	....	Mixed—osteoarthritis, rheumatoid; also hypertensive cardiovascular disease	IV
54	....	16	Rheumatoid	IV
61	9	....	Rheumatoid	III
35	7	6	Marie-Strumpell	I
41	12	....	Marie-Strumpell	II
58	8	....	Rheumatoid	III
40	6	....	Osteoarthritis	I
53	4	....	Osteoarthritis; also chronic sinusitis	II
56	10	....	Osteoarthritis; also severe occipital headache, etiology unknown	II
37	14	....	Rheumatoid	I
43	3	....	Osteoarthritis	II
31	7	....	Marie-Strumpell	II
40	6	....	Osteoarthritis	I
40	7	....	Osteoarthritis	I
37	4	6	Osteoarthritis	I
60	3	....	Osteoarthritis	II
55	31	....	Rheumatoid	III
31	2	6	Rheumatoid; also severe scleroderma and Raynaud's disease	I
62	20	....	Rheumatoid	III
39	5	....	Rheumatoid	II
37	4	....	Osteoarthritis	II
52	4	....	Mixed—osteoarthritis, rheumatoid	II
22	....	8	Rheumatoid	III
38	5	....	Osteoarthritis; also scoliosis of entire spine	II
53	33	....	Osteoarthritis	II
68	31	....	Osteoarthritis; also arteriosclerotic heart disease	III
21	10	....	Osteoarthritis	II

*The patients were classified as to functional capacity in accordance with criteria for rheumatoid arthritis adopted by the American Rheumatism Association[20]: Class I, capable of usual activities, without handicap; II, adequate for normal activities despite discomfort or limited motion; III, limited; capable of little or none of usual activities or self-care; IV, largely or wholly incapacitated; in wheel chair or bed, little or no self-care.

Fifteen of the patients were discharged from the hospital and given the agents or the placebo on a different dosage schedule than had been followed in the hospital, for a period ranging from 13 to 65 additional days, with no restriction as to activities or diet.

The "daily report card" technique described by Greiner and co-workers,[14] slightly modified, was used as a continuous subjective estimate of clinical effect. The reports were analyzed statistically, both weekly and cumulatively.

The following methods were used to evaluate clinically the extent of the disease before, during, and after the treatment period: (1) Clinical history-taking and physical examination; (2) goniometry of affected joints; (3) cinematography; (4) weekly clinical appraisal by the physician; (5) weekly weight record; (6) daily temperature and pulse records.

Laboratory studies on each patient before and after the treatment period included: (1) hemogram; (2) urinalysis; (3) weekly determination of sedimentation rate (Cutler method); (4) roentgenograms of affected joints; (5) electrocardiogram (limb and CF_4 leads); (6) determination of basal metabolic rate; (7) bromsulfalein liver function test (5 mg. per kg., 45-minute test); (8) cephalin cholesterol flocculation liver function test (Hanger); (9) determination of total serum protein and albumin-globulin ratios; (10) weekly counts of eosinophils (Forsham and Thorn technique).

Additional chemical studies were made on five patients in the control group and on five patients in the treated group, for further appraisal of the possible metabolic effects of the agents being tested.

Various determinations of urine content were made on specimens from continuous three-day collections, and determinations on blood samples were made biweekly. These determinations and the analytical methods used are briefly outlined as follows:

1. *Glycocyamine*—The method described by Dubnoff and Borsook[7] was used for determination of glycocyamine in blood and urine. Blood was deproteinized with sodium tungstate and sulfuric acid, the precipitate was removed by centrifugation, and the supernatant fluid passed through an 85 mm. permutit column to remove arginine. The permutit column was washed, and a sample of this filtrate was submitted to colorimetry. Color was developed by the Sakaguchi reaction[23] using alpha naphthol, urea, and hypobromite, added in the order named, at 4° C. Analysis of urine for glycocyamine followed the same procedure except that deproteinization was not required.

2. *Creatine and Creatinine*—Folin's method,[9] utilizing the Jaffe reaction, was used on both plasma and urine in the determination of creatinine content. Creatine content was determined by difference after conversion to creatinine by boiling with acid.

3. *Nitrogen*—Determination of urinary nitrogen was accomplished using standard Kjeldahl procedures, the digestion being carried out with sulfuric acid and hydrogen peroxide. Ammonia thus formed was determined by distillation into standard sulfuric acid and the excess titrated with standard alkali.

4. *Urinary Urea Nitrogen*—Diluted urine was passed through a permutit column to remove ammonia, then treated with hypobromite in the Van Slyke-Neill apparatus, and the liberated nitrogen measured gasometrically.[15, 19, 21]

5. *Total Plasma Proteins and Albumin*—The colorimetric method described by Greenberg[13] was used—heating diluted plasma with alkali and development of color with the phenol reagent of Folin and Ciocalteu.[10] Albumin was determined by removal of globulin with 22.5 percent sodium sulfate. The filtrate containing the albumin was then treated in the same manner as in the total protein determination.

6. *Sodium and Potassium in Urine and Plasma*—The internal standard flame photometer was used to determine these metals in both urine and plasma.

7. *Urinary Calcium* — The method of Michaels and co-workers[17] as applied to urine, was found satisfactory, with slight modification. Calcium was precipitated as calcium oxalate. This precipitate was redissolved and calcium was again precipitated, this time as the phosphate, which, after separation, was dissolved in hydrochloric acid and the phosphate content determined by the method of Fiske and Subarrow.[8]

8. *Plasma Calcium*—The method of Clark and Collip[5] was used. Calcium was precipitated directly from serum with ammonium oxalate and the oxalate salt was then titrated with potassium permanganate.

9. *Serum and Urine Phosphorus*—A trichloracetic filtrate of serum was treated with sulfuric acid and sodium molybdate to form phosphomolybdate. This was reduced with stannous chloride to give a blue color which was measured colorimetrically.[16]

The method of Fiske and Subarrow[8] was used in the analysis of urine for phosphorus. The same principle described above was applied, except that the reducing agent was 1, 2, 4-aminonaphtholsulfonic acid.

10. *Uric Acid in Plasma and Urine*—The method described by Folin[11] in 1934 was used. This was based on the reducing action of uric acid upon a phosphotungstic acid reagent which produces a blue color. The procedure was carried out on a filtrate of plasma, and directly on urine.

11. *Alkaline Phosphatase*—Serum was incubated with buffered glycerophosphate at pH 8.6 after the method of Bodansky.[1] The inorganic phosphate which was liberated was then determined as described earlier in this presentation.

12. *17-Ketosteroids*—Urine was heated with hydrochloric acid and extracted with ether after the procedure described by Drekter and co-workers.[6] A sample of the ether extract was then evaporated and the residue was subjected to the Zimmerman reaction[15] for the colorimetric determination of dehydroisoandrosterone.

RESULTS

A. *General Observations on All Patients*

On analysis of the "daily report card" data of the hospitalized patients, no significant difference in relief of pain was apparent between the treated and the control groups. The "daily report card" data on the 15 patients (8 treated, 7 controls) on whom study was continued after their discharge from hospital indicated a slight advantage for the treated group; however, study of all the data on the 15 patients, covering both the in-patient and out-patient periods, revealed very little advantage, if any, ascribable to the agents under test.

To ascertain the effect of the agents on any one specific type of arthritis, an analysis was made of "daily report card" data for certain patients with well-defined clinical varieties of arthritis. No significant difference in relief of pain was apparent between the control and the treated groups.

Clinical results after treatment of each patient on the in-patient study are summarized in Table 2. In this appraisal the investigators had at hand for comparison all the clinical laboratory data, the "daily report card" data, goniometry of the affected joints before and after treatment, and clinical observations on each patient. No significant difference was apparent from the clinical evaluation of treated and control groups.

Comparison of goniometric findings prior to and subsequent to the treatment period indicated that there was no trend in either the treated or the control group. Motion pictures taken before and after treatment showed no advantage for either group of patients. No significant alteration in weight or in temperature was observed in either group.

The sedimentation rate was determined weekly by the Cutler method for each patient. Sedimentation rates increased or decreased in various patients from both the test and control groups, but there was no uniform trend in either group.

Follow-up roentgenograms, electrocardiograms, determination of basal metabolic rate, bromsulfalein and cephalin cholesterol flocculation tests, and determination of serum protein content were completed only on patients in the earliest groups of 18 patients—9 treated and 9 controls. A study of these data indicated that there was no significant change in any patient. It was therefore decided that no information could be gained by repeating these time-consuming examinations on the remainder of the patients. All roentgenograms on these patients were interpreted by a radiologist and in no patient was there reported any alteration in the appearance of the affected joints.

Weekly eosinophil counts by the Forsham and Thorn technique were done on all patients included in the study. Analysis of the accumulated data indicated that there was no significant alteration during the course of in-patient observation.

B. *Special Chemical Studies on Ten Patients*

Extensive chemical studies were carried out on ten patients, five treated and five controls. These studies were designed to investigate the mechanism of action of glycocyamine and betaine should these agents prove to be effective in arthritis. All blood tests were done on venous samples taken after an

TABLE 2.—*Summary of Final Evaluation by Physician of Patients on In-Patient Study.*

	Clinical Appraisal								Number of Patients
	No Change		Slight Improvement		Moderate Improvement		Marked Improvement		
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	
Controls	12	54.5	6	27.3	4	18.2	0	0	22
Treated patients	14	63.6	4	18.2	2	9.1	2	9.1	22

overnight fast. Urinalyses were done on samples from pooled three-day collections. In all cases treatment or placebo administration was begun on the sixth day of study.

The five patients who received glycocyamine began to excrete large amounts of the drug in the urine soon after treatment was started. In three cases glycocyamine excretion began to decrease about the thirtieth day of treatment and trended downward for the remainder of the period. The explanation for this is not apparent. This phenomenon was not accompanied by a corresponding increase in creatine or creatinine excretion or by increased amounts of glycocyamine, creatine or creatinine in the blood. However, nitrogen balance data indicated that these patients were retaining larger amounts of nitrogen than they had retained earlier in the experiment. This observation suggested that the metabolic disposition of administered glycocyamine had changed so that a greater proportion of the material was stored or was altered in such a manner that it did not appear in the urine. It is also a possibility that absorption from the gastrointestinal tract was reduced to such an extent that much of the ingested glycocyamine was destroyed or excreted unchanged in the feces. As no stool analyses were done during this study, this possibility cannot be confirmed. Urinary excretion of glycocyamine in the control group was consistently low throughout the experiment.

Glycocyamine blood levels were determined for both treated and control groups, and there was no significant difference between the two. This is attributed to the fact that the blood specimens were taken in the morning, after a 12-hour fast, and some 12 hours after administration of glycocyamine. Unpublished data from records in the hospital indicate that in about six hours after the oral administration of 5 gm. of glycocyamine with betaine the concentration of glycocyamine in the blood has returned to the preingestion level.

The excretion of creatine and creatinine was notably increased in all patients who received glycocyamine and betaine. This increase was expected, since these agents are known to be creatine precursors, and it clearly indicated the conversion of glycocyamine into creatine. Blood levels of creatine and creatinine, however, were not significantly different between the treated and the control groups, a finding which was not unexpected in view of the ready elimination of these substances by the kidneys.

Urinary excretion of nitrogen was at a higher level in the treated patients than in the controls. This difference is largely accounted for by nitrogen originating from substances other than urea. Glycocyamine probably accounted for most of this extra nitrogen.

Of the ten patients selected for metabolic studies, those in the treated group all stored nitrogen consistently, as expected, but had a tendency to increased storage in the last twelve to fifteen days of the study in three of the cases. This increase occurred at a time when glycocyamine excretion in the urine was decreasing, as was pointed out earlier in this presentation. More variability in nitrogen balance was observed in the four control patients, two of them having persistent positive nitrogen balance while the remaining two had pronounced variability.

Serum protein content changed little or not at all during the study and was practically identical for treated and control groups.

If clinical improvement had been noted after the administration of glycocyamine and betaine, the influence of the adrenal gland in this effect would be of interest. Urinary excretion of sodium and potassium, which is a good index of adrenocortical activity, and the plasma levels of these elements were therefore studied. There was no significant difference between the two groups in either urinary excretion or plasma content of sodium and potassium.

Because of the reported change in bony abnormalities in rheumatoid arthritis during treatment with glycocyamine and betaine, the urinary excretion of calcium and phosphorus and the content of these elements in the blood, were studied. While the treated patients excreted daily more calcium than did patients in the control group, this cannot be attributed to the treatment because there is no change apparent after institution of therapy. In general, there appears to be no difference between the two groups in phosphorus excretion or in content of calcium or phosphorus in the blood, nor in content of alkaline phosphatase in the serum.

Uric acid content in the urine and in the plasma of treated patients was practically identical with that in patients in the control group throughout the experiment. One patient, in the control group, with arthritis of the gouty type, had a persistently high level of uric acid in the blood.

As it had been reported[2] that patients given glycocyamine and betaine were likely to have periods of hypoglycemia, the level of sugar in the blood was regularly observed during this study. Once again there was no detectable difference between the treated and control groups although in both groups, for no apparent reason, a slight downward trend was confirmed during the study. Diet may have been the causative factor.

Determinations of urinary 17-ketosteroids were made regularly in the first eighteen days of the study. There was no apparent deviation from normal in either treated or control patients.

CONCLUSION

Under the conditions of the study here reported, no significant advantage has been shown to result

from oral administration of glycocyamine and betaine to patients with arthritis of various types.

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